

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:	:	
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Ralf WOLLESCHENSKY	:	
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SEPARATING EXCITATION	:	
LIGHT AND EMISSION LIGHT IN	:	
A MICROSCOPE	:	

**CLAIMS MARKED TO SHOW CHANGES**

**In the Specification**

(Another version of any replacement paragraph(s) or section heading(s) must be provided, on one or more pages separate from the amendment, marked up to show all the changes relative to the previous version of the paragraph(s). The changes may be shown by brackets (for deleted matter) or underlining (for added matter), or by any equivalent marking system. A marked up version does not have to be supplied for an added paragraph or a deleted paragraph as it is sufficient to state that a particular paragraph has been added, or deleted. See 37 CFR § 1.121.)

The paragraph at page \_\_, lines \_\_ has been amended as follows:

**In the Claims**

Claims 1-11 (including the claims numbered 2a and 3a) have been canceled without prejudice or disclaimer.

New claims 12-65 have been added as follows:

12. A microscope having a microscope beam path and including a light diffracting element for the separation of excitation light and emission light in the microscope beam path.

13. The microscope of claim 12, wherein the microscope is a laser scanning microscope.

14. The microscope of claim 12, wherein the light diffracting element is traversed both by the excitation light and the emission light.

15. The microscope of claim 14, wherein the light emitted by a sample comprises fractions of the excitation light and of wavelength-shifted fluorescence fractions.

16. The microscope of claim 12, wherein the light diffracting element influences at least one excitation wavelength by diffraction, whereas other wavelengths emitted by a sample pass in uninfluenced form through the element and are thereby spatially separated from the excitation light.

17. The microscope of claim 13, further including means for switching the light diffracting element by way of a frequency change from a first wavelength of a first laser to a second wavelength of a second laser.

18. The microscope of claim 12, further including at least one optical element influencing the light direction provided in the excitation beam path upstream of the element and/or in the detection beam path downstream of the element in order to improve light fraction separation.

19. The microscope of claim 12, wherein the light diffracting element comprises an AOTF.

20. The microscope of claim 12, wherein the optical element comprises a reflection element.

21. The microscope of claim 12, wherein the optical element comprises a light refracting element

22. A microscope having a microscope beam path and including a light diffracting element for the separation of excitation light and emission light in the microscope beam path and for regulating the excitation intensity.

23. The microscope of claim 22, wherein the microscope is a laser scanning microscope.

24. A microscope having a microscope beam path and including a plurality of light diffracting element for the separation of excitation light and emission light in the microscope beam path and for simultaneously or individually feeding in different wavelengths.

25. The microscope of claim 24, wherein the microscope is a laser scanning microscope.

26. The microscope of claim 24, wherein the light detecting elements comprise firstly an AOTF and then an AOM in the direction of the detection.

27. The microscope of claim 24, wherein at least one of an AOTF and an AOM are used as light diffracting elements.

28. A fluorescence microscope comprising:

a radiation source (L1, L2, L3) for irradiating a sample,

a detection device (DE, DT, NFT) for the detection of emission light emitted by the sample,

microscope optics for directing excitation light to the sample and for directing emission light back in the direction of the radiation source and detection device,

an acousto-optical element (AOM, AOTF) for diffracting excitation light and with which it is possible to regulate an intensity of the diffracted excitation light, the acousto-optical element being positioned between the radiation source and microscope optics in such a way that diffracted excitation light can be introduced into the microscope optics (SC1, SC2, SCO, M1), wherein:

the emission light emitted by the sample has fractions of excitation light and fractions of wavelength-shifted fluorescence light,

excitation light emitted by the sample can be deflected in the direction of the radiation source by the acousto-optical device (AOM, AOTF), and

wavelength-shifted fluorescence light emitted by the sample can be transmitted undiffracted through the acousto-optical element (AOM, AOTF) and is spatially separable from excitation light fractions of the emission light, and wherein:

the detection device (DE, DT, NFT) is so positioned with respect to the acousto-optical element that wavelength-shifted fluorescence light transmitted undiffracted through the acousto-optical element (AOM, AOTF) is detectable by means of the detection device (DE, DT, NFT) and

further comprising a filter device (LF), which for the selective detection of wavelength-shifted fluorescence light in the detection device (DE, DT, NFT) is located between the acousto-optical element and the detection device (DE, DT, NFT).

29. The fluorescence microscope of claim 28, wherein the fluorescence microscope is a confocal fluorescence laser microscope.

30. The fluorescence microscope of claim 28, wherein the radiation source is a laser emitting excitation light.

31. The fluorescence microscope of claim 28, further comprising at least one optical element influencing the light direction provided in at least one of an excitation beam path upstream of the acousto-optical element (AOM, AOTF) and a detection beam path downstream of the acousto-optical element (AOM, AOTF) for the improved separation of the light fractions.

32. The fluorescence microscope of claim 31, wherein the optical element comprises a reflection element (S1, S2, PS, S) selected from the group consisting of a mirror (S), a bimirror (S1, S2) and a vapourized prism (PS).

33. The fluorescence microscope of claim 31, wherein the optical element comprises a light refracting element (P) which is located in at least one of an excitation beam path upstream of the acousto-optical element (AOM, AOTF) and a detection beam path downstream of the acousto-optical element (AOM, AOTF).

34. The fluorescence microscope of claim 33, wherein the light refracting element comprises an unvapourized prism (P).

35. The fluorescence microscope of claim 32, further comprising a further optical element comprising a light refracting element (P) which is located in at least one of an excitation beam path upstream of the acousto-optical element (AOM, AOTF) and a detection beam path downstream of the acousto-optical element (AOM, AOTF).

36. The fluorescence microscope of claim 35, wherein the light refracting element comprises an unvapourized prism (P).

37. A fluorescence microscope, comprising:

a radiation source (L1, L2, L3) which emits excitation light for irradiating a sample,

a detection device (DE, DT, NFT) for the detection of emission light emitted by the sample,

microscope optics for directing excitation light to the sample and for directing emission light back in the direction of the radiation source and detection device,

an acousto-optical element (AOM, AOTF) for diffracting excitation light and which is positioned between the radiation source and microscope optics in such a way that diffracted excitation light can be introduced into the microscope optics (SC1, SC2, SCO, M1), wherein:

the emission light emitted by the sample has fractions of excitation light and fractions of wavelength-shifted fluorescence light,

excitation light emitted by the sample can be deflected in the direction of the radiation source by diffraction by the acousto-optical device (AOM, AOTF), and

wavelength-shifted fluorescence light emitted by the sample can be transmitted undiffracted through the acousto-optical element (AOM, AOTF) and is spatially separable from excitation light fractions of the emission light, and wherein:

the detection device (DE, DT, NFT) is so positioned with respect to the acousto-optical element that wavelength-shifted fluorescence light transmitted undiffracted through the acousto-optical element (AOM, AOTF) can be detected by means of the detection device (DE, DT, NFT), and further comprising:



a filter device (LF), which for the selective detection of wavelength-shifted fluorescence light in the detection device (DE, DT, NFT) is positioned between the acousto-optical element and the detection device (DE, DT, NFT), and

at least one light reflecting element (P) for influencing the light direction and for separating the light fractions, which is located in at least one of an excitation beam path upstream of the acousto-optical element (AOM, AOTF) and a detection beam path downstream of the acousto-optical element (AOM, AOTF).

38. The fluorescence microscope of claim 37, wherein the fluorescence microscope is a confocal fluorescence laser microscope.

39. The fluorescence microscope of claim 37, wherein the radiation source (L1, L2, L3) is a laser.

40. The fluorescence microscope of claim 37, wherein the at least one light reflecting element (P) is an unvapourized prism (P).

41. The fluorescence microscope of claim 28, wherein the acousto-optical elements (AOM, AOTF) comprise firstly an AOM and then an AOTF in the direction of the microscope optics (SC1, SC2, SCO, M1).

42. The fluorescence microscope of claim 37, wherein the acousto-optical elements (AOM, AOTF) comprise firstly an AOM and then an AOTF in the direction of the microscope optics (SC1, SC2, SCO, M1).

43. A fluorescence microscope, comprising:  
a radiation source (L1, L2, L3) which emits excitation light for irradiating a sample,  
a detection device (DE, DT, NFT) for the detection of emission light emitted by the sample,

microscope optics for directing excitation light to the sample and for directing emission light back in the direction of the radiation source and detection device,

a plurality of acousto-optical elements (AOM, AOTF) for diffracting excitation light, which are so positioned between the radiation source and the microscope optics that diffracted excitation light can be introduced into the microscope optics (SC1, SC2, SCO, M1), wherein:

in the direction of the microscope optics (SC1, SC2, SCO, M1) as acousto-optical elements (AOM, AOTF) are firstly provided an AOM and then an AOTF,

the emission light emitted by the sample has fractions of excitation light and fractions of wavelength-shifted fluorescence light,

excitation light emitted by the sample is deflectable by diffraction in the direction of the radiation source by the acousto-optical devices (AOM, AOTF), and

wavelength-shifted fluorescence light emitted by the sample can be transmitted undiffracted through the acousto-optical elements (AOM, AOTF) and is spatially separable from excitation light fractions of the emission light, and wherein:

the detection device (DE, DT, NFT) is so positioned with respect to the acousto-optical elements that wavelength-shifted fluorescence light transmitted undiffracted through the acousto-optical elements (AOM, AOTF) is detectable by means of the detection device (DE, DT, NFT), and further comprising:

a filter device (LF), which for the selective detection of wavelength-shifted fluorescence light in the detection device (DE, DT, NFT) is positioned between the acousto-optical elements and the detection device (DE, DT, NFT).

44. The fluorescence microscope of claim 43, wherein the fluorescence microscope is a confocal fluorescence laser microscope.

45. The fluorescence microscope of claim 43, wherein the radiation source (L1, L2, L3) is a laser.

46. The fluorescence microscope of claim 28, wherein at least one glass fibre is provided for feeding in excitation light.

47. The fluorescence microscope of claim 37, wherein at least one glass fibre is provided for feeding in excitation light.

48. The fluorescence microscope of claim 43, wherein at least one glass fibre is provided for feeding in excitation light.

49. The fluorescence microscope of claim 43, further comprising at least one optical element influencing the light direction provided in at least one of an excitation beam path upstream of the acousto-optical element (AOM, AOTF) and a detection beam path downstream of the acousto-optical element (AOM, AOTF) to bring about improved separation of the light fractions.

50. The fluorescence microscope of claim 28, wherein:  
the radiation source (L1, L2, L3) is constructed as a plurality of lasers (L1, L2, L3) having different wavelengths,  
a plurality of the acousto-optical elements (AOM, AOTF) are provided and with each laser (L1, L2, L3) is associated at least one acousto-optical element (AOM, AOTF),  
the different wavelengths by diffraction in the acousto-optical elements (AOM, AOTF) can be simultaneously or individually fed into a microscope beam path (SC1, SC2, SCO, M1), and

wavelength-shifted emission light and excitation light having in each case a different wavelength can be transmitted undiffracted through the respective acousto-optical elements (AOM, AOTF).

51. The fluorescence microscope of claim 28, wherein the acousto-optical elements comprise at least one of an AOTF and an AOM.

52. The fluorescence microscope of claim 50, wherein the excitation power of each laser (L1, L2, L3) is independently adjustable with the respective acousto-optical element (AOM, AOTF).

53. The fluorescence microscope of claim 30, wherein the acousto-optical elements (AOM, AOTF) can be switched by a frequency change from a first wavelength of a first laser to a second wavelength of a second laser.

54. The fluorescence microscope of claim 28, wherein the excitation light can be introduced into the microscope optics (SC1, SC2, SCO, M1) by diffraction at the acousto-optical element (AOM, AOTF) in the first diffraction order.

55. The fluorescence microscope of claim 28, further comprising an excitation and detection pinhole (PH) located upstream of the microscope optics (SC1, SC2, SCO, M1).

56. The fluorescence microscope of claim 50, wherein the radiation of the plurality of lasers (L1, L2, L3) in the direction of the microscope optics (SC1, SC2, SCO, M1) can be successively fed into the microscope beam path in a sequence based on decreasing wavelength.

57. The fluorescence microscope of claim 28, wherein at least one of UV light, visible light and infrared light can be fed into the microscope beam path.

58. A device for feeding light into a beam path of a microscope, comprising:  
a plurality of light sources (L1, L2, L3), which emit light of different wavelengths,  
wherein:  
a plurality of light diffracting elements is provided, the light diffracting elements being located on a common optical axis for combining the light of the plurality of light sources (L1, L2, L3), and  
at least one light diffracting element associated is with each light source (L1, L2, L3),  
and wherein the different wavelengths by diffraction in the light diffracting elements can be simultaneously or individually fed into the common optical axis and are combinable in the common optical axis.

59. The device of claim 58, wherein the microscope is a confocal fluorescence laser microscope.

60. The device of claim 58, wherein the plurality of light diffracting elements comprise acousto-optical elements (AOM, AOTF).

61. The device of claim 58, wherein the light diffracting elements are chosen from the group consisting of an AOTF and an AOM.

62. The device of claim 61, wherein the acousto-optical elements (AOM, AOTF) comprise firstly an AOM and then an AOTF in the direction of the microscope optics (SC1, SC2, SCO, M1).

63. The microscope of claim 12, wherein the microscope is a confocal microscope.

64. The microscope of claim 22, wherein the microscope is a confocal microscope.

65. The microscope of claim 24, wherein the microscope is a confocal microscope.